C. Expression and Production of a Double-Stranded RNA Target in Two Strains of *Escherichia coli*: (1) AB309-105, and, (2) BL21(DE3)

[0464] The procedures described below are followed in order to express suitable levels of fungal double-stranded RNA of fungal target in bacteria. An RNaseIII-deficient strain, AB309-105, is used in comparison to wild-type RNaseIII-containing bacteria, BL21(DE3).

Transformation of AB309-105 and BL21(DE3)

[0465] Three hundred ng of the plasmid are added to and gently mixed in a 50 μl aliquot of ice-chilled chemically competent *E. coli* strain AB309-105 or BL21(DE3). The cells are incubated on ice for 20 minutes before subjecting them to a heat shock treatment of 37° C. for 5 minutes, after which the cells are placed back on ice for a further 5 minutes. Four hundred and fifty μl of room temperature SOC medium is added to the cells and the suspension incubated on a shaker (250 rpm) at 37° C. for 1 hour. One hundred μl of the bacterial cell suspension is transferred to a 500 ml conical flask containing 150 ml of liquid Luria-Bertani (LB) broth supplemented with 100 μg/ml carbenicillin antibiotic. The culture is incubated on an Innova 4430 shaker (250 rpm) at 37° C. overnight (16 to 18 hours).

Chemical Induction of Double-Stranded RNA Expression in AB309-105 and BL21(DE3)

[0466] Expression of double-stranded RNA from the recombinant vector, pGBNJ003, in the bacterial strain AB309-105 or BL21(DE3) is made possible since all the genetic components for controlled expression are present. In the presence of the chemical inducer isopropylthiogalactoside, or IPTG, the T7 polymerase will drive the transcription of the target sequence in both antisense and sense directions since these are flanked by oppositely oriented T7 promoters.

[0467] The optical density at 600 nm of the overnight bacterial culture is measured using an appropriate spectrophotometer and adjusted to a value of 1 by the addition of fresh LB broth. Fifty ml of this culture is transferred to a 50 ml Falcon tube and the culture then centrifuged at 3000 g at 15° C. for 10 minutes. The supernatant is removed and the bacterial pellet resuspended in 50 ml of fresh S complete medium (SNC medium plus 5 μg/ml cholesterol) supplemented with 100 μg/ml carbenicillin and 1 mM IPTG. The bacteria are induced for 2 to 4 hours at room temperature.

Heat Treatment of Bacteria

[0468] Bacteria are killed by heat treatment in order to minimise the risk of contamination of the artificial diet in the test plates. However, heat treatment of bacteria expressing double-stranded RNA is not a prerequisite for inducing toxicity towards the insects due to RNA interference. The induced bacterial culture is centrifuged at 3000 g at room temperature for 10 minutes, the supernatant discarded and the pellet subjected to 80° C. for 20 minutes in a water bath. After heat treatment, the bacterial pellet is resuspended in 1.5 ml MilliQ water and the suspension transferred to a microfuge tube. Several tubes are prepared and used in the bioassays for each refreshment. The tubes are stored at −20° C. until further use.

SEQUENCE LISTING

The patent application contains a lengthy "Sequence Listing" section. A copy of the "Sequence Listing" is available in electronic form from the USPTO web site (http://seqdata.uspto.gov/?pageRequest=docDetail&DocID=US20150065557A1). An electronic copy of the "Sequence Listing" will also be available from the USPTO upon request and payment of the fee set forth in 37 CFR 1.19(b)(3).

What is claimed is:

1. An isolated polynucleotide sequence selected from the group consisting of a polynucleotide sequence comprising a nucleic acid sequence set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 49-158, 159, 160, 163, 168, 173, 178, 183, 188, 193, 198, 203, 208, 215, 220, 225, 230, 247, 249, 251, 253, 255, 257, 259, 275-472, 473, 478, 483, 488, 493, 498, 503, 513, 515, 517, 519, 521, 533-575, 576, 581, 586, 591, 596, 601, 603, 605, 607, 609, 621-767, 768, 773, 778, 783, 788, 793, 795, 797, 799, 801, 813-862, 863, 868, 873, 878, 883, 888, 890, 892, 894, 896,908-1040, 1041, 1046, 1051, 1056, 1061, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1161-1571, 1572, 1577, 1582, 1587, 1592, 1597, 1602, 1607, 1612, 1617, 1622, 1627, 1632, 1637, 1642, 1647, 1652, 1657, 1662, 1667, 1672, 1677, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1730-2039, 2040, 2045, 2050, 2055, 2060, 2065, 2070, 2075, 2080, 2085, 2090, 2095, 2100, 2102, 2104, 2106, 2108, 2120-2338, 2339, 2344, 2349, 2354, 2359, 2364, 2366, 2368, 2370, 2372, 2384-2460, 2461, 2466, 2471, 2476 and 2481; a polynucleotide sequence having at least 70% sequence identity to a nucleic acid sequence set forth in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 49-158, 159, 160, 163, 168, 173, 178, 183, 188, 193, 198, 203, 208, 215, 220, 225, 230, 24 7' 249, 251, 253, 255, 257, 259, 275-472, 473, 478, 483, 488, 493, 498, 503, 513, 515, 517, 519, 521, 533-575, 576, 581, 586, 591, 596, 601, 603, 605, 607, 609, 621-767, 768, 773, 778, 783, 788, 793, 795, 797, 799, 801, 813-862, 863, 868, 873, 878, 883, 888, 890, 892, 894, 896, 908-1040, 1041, 1046, 1051, 1056, 1061, 1071, 1073, 1075, 1077, 1079, 1081, 1083, 1085, 1087, 1089, 1091, 1093, 1095, 1097, 1099, 1101, 1103, 1105, 1107, 1109, 1111, 1113, 1161-1571, 1572, 1577, 1582, 1587, 1592, 1597, 1602, 1607, 1612, 1617, 1622, 1627, 1632, 1637, 1642, 1647, 1652, 1657, 1662, 1667, 1672, 1677, 1682, 1684, 1686, 1688, 1690, 1692, 1694, 1696, 1698, 1700, 1702, 1704, 1730-2039, 2040, 2045, 2050, 2055, 2060, 2065, 2070, 2075, 2080, 2085, 2090, 2095, 2100, 2102, 2104, 2106,